AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

LETTER TO THE EDITOR

The *Pseudomonas aeruginosa* Population among Cystic Fibrosis Patients in Quebec, Canada: a Disease Hot Spot without Known Epidemic Isolates

Julie Jeukens,[a] Luca Freschi,[a] Irena Kukavica-Ibrulj,[a] Jean-Guillaume Emond-Rheault,[a] Christian Allard,[b] Jean Barbeau,[c] André Cantin,[d] Steve J. Charette,[a,e] Eric Déziel,[f] François Malouin,[d] Julie Milot,[e] Dao Nguyen,[g] Clara Popa,[h] Brian Boyle,[a] Roger C. Levesque[a]

[a]Institute for Integrative and Systems Biology (IBIS), Université Laval, Quebec City, QC, Canada
[b]CSSS de Chicoutimi, Chicoutimi, QC, Canada
[c]Université de Montréal, Montreal, QC, Canada
[d]Université de Sherbrooke, Sherbrooke, QC, Canada
[e]Institut universitaire de cardiologie et de pneumologie de Québec (IUCPQ), Quebec City, QC, Canada
[f]INRS Institut Armand-Frappier, Laval, QC, Canada
[g]McGill University, Montreal, QC, Canada
[h]Centre intégré de santé et de services sociaux de l'Abitibi-Témiscamingue, Hôpital de Rouyn-Noranda, Rouyn-Noranda, QC, Canada

KEYWORDS *Pseudomonas aeruginosa*, clonal strains, cystic fibrosis, epidemic strains, genomes, genomics, molecular epidemiology

The Canadian province of Quebec has prevalence rates of cystic fibrosis (CF) that are among the highest in the world, with an average of 1 in 2,500 newborns (1; http://www.cysticfibrosis.ca), and up to 1 in 902 in the region of Saguenay–Lac-Saint-Jean (2–4). Still, unlike in other provinces (5–7), molecular epidemiology data are not available for the most common respiratory pathogen associated with this disease, *Pseudomonas aeruginosa* (8). A recent national molecular typing study included isolates from two clinics in Montreal, the largest city in Quebec (9), but analyses were not directed toward investigating each province individually. Here, we sought to describe the population structure of *P. aeruginosa* in Quebec to improve the epidemiological basis for infection control and patient management. We were mainly interested in the prevalences of epidemic strains, which have been reported in the Prairie Provinces and Ontario and are generally associated with worse clinical prognoses (7, 9, 10).

We selected all sequenced Quebec isolates from the International *Pseudomonas* Consortium Database (http://ipcd.ibis.ulaval.ca) (11) and 11 reference strains (Data Set S1). The final data set of 298 genomes comprised isolates from five CF clinics scattered across southern Quebec, as well as from environmental sources. We performed a core genome phylogenetic analysis with SaturnV (https://github.com/ejfresch/saturnV) (12), and produced *in silico* molecular typing using Short Read Sequence Typing for Bacterial Pathogens (SRST2) v0.2.0 (13).

No geographic structure emerged from the five CF clinics represented (Fig. 1). However, multiple clones were shared among two or more clinics. Based on molecular sequence typing (14; https://pubmlst.org/), the most pervasive clones (sequence type 17 [ST17], ST155, and ST179), including well-characterized clone C (15), are all widely distributed around the world and likely reflect environmental abundance rather than patient-to-patient transmission (16). This is further supported by the presence of environmental isolates, which incidentally came from hospital sinks (Data Set S1), within ST155 and ST179. Encouragingly, not a single isolate in this study corresponded to epidemic strains identified in Ontario (Liverpool epidemic strain and epidemic strain B [7]) or the Prairies (Prairie epidemic strain [PES; ST192] [5]). It is not clear whether this is due to

Citation Jeukens J, Freschi L, Kukavica-Ibrulj I, Emond-Rheault J-G, Allard C, Barbeau J, Cantin A, Charette SJ, Déziel E, Malouin F, Milot J, Nguyen D, Popa C, Boyle B, Levesque RC. 2019. The *Pseudomonas aeruginosa* population among cystic fibrosis patients in Quebec, Canada: a disease hot spot without known epidemic isolates. J Clin Microbiol 57:e02019-18. https://doi.org/10.1128/JCM.02019-18.

Editor Geoffrey A. Land, Carter BloodCare & Baylor University Medical Center

Address correspondence to Roger C. Levesque, rclevesq@ibis.ulaval.ca.

Accepted manuscript posted online 3 April 2019
Published 24 May 2019

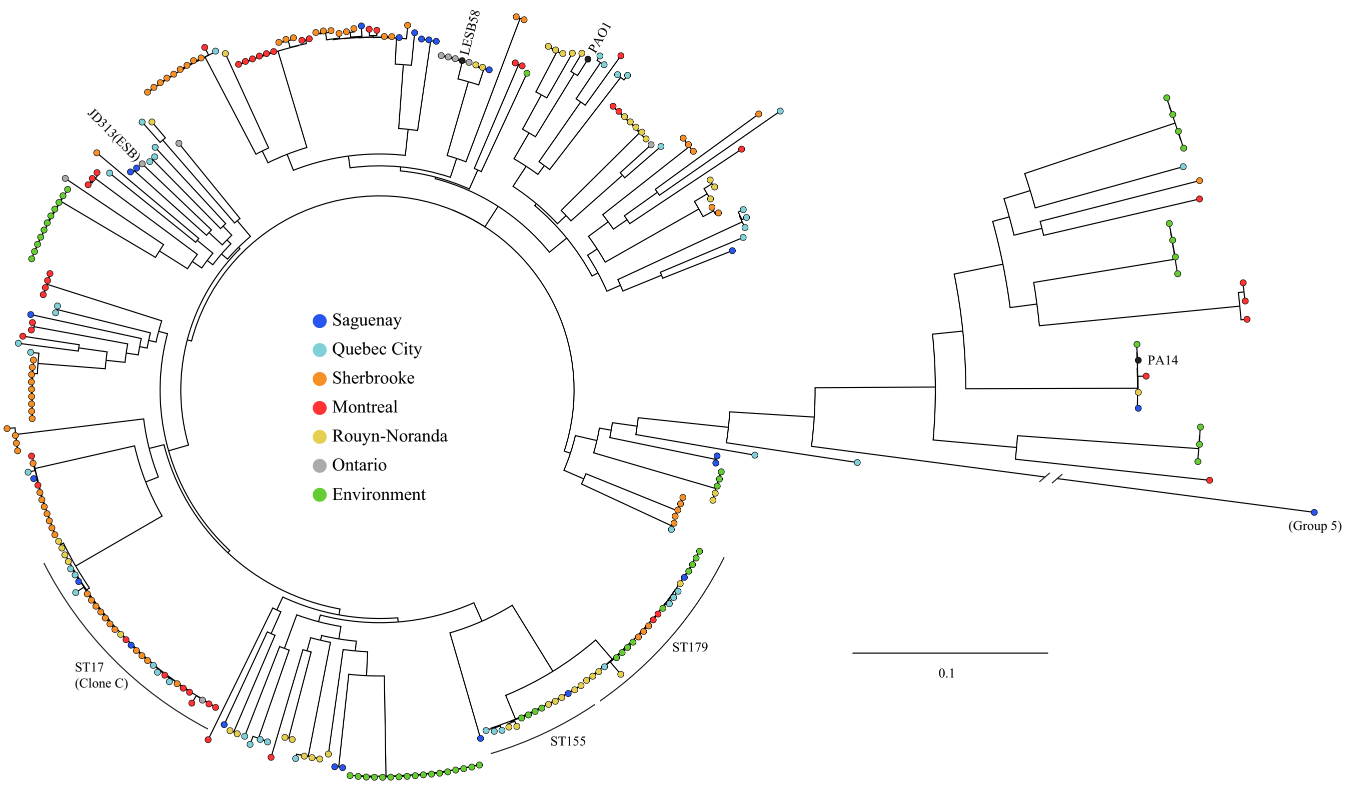

FIG 1 Phylogenetic tree of 298 *P. aeruginosa* isolates (66,805 SNPs, 1,000 bootstraps). Three refence isolates are labeled and represented in black (LESB58 is the reference for the Liverpool epidemic strain). Ontario's epidemic strain B (ESB) is also labeled. The most pervasive sequence types (STs) are identified. Group 5 isolates are relatively rare among CF patients (21). The same tree with genome IDs, detailed STs, and bootstrap values is provided in Fig. S1. Most environmental isolates are from hospitals and dental clinics of the greater Montreal area. Intrapatient redundancy, i.e., identical strains from the same patient, was not removed (numbers of patients: Quebec City, $n = 33$; Rouyn-Noranda, $n = 31$; Montreal, $n = 27$; Sherbrooke, $n = 20$; and Saguenay, $n = 17$). Isolates from the same clinic spanned up to a year for Montreal, Quebec City, and Saguenay, 3 years for Sherbrooke, and 6 years for Rouyn-Noranda; all clinical isolates were collected between 2007 and 2016 (see Data Set S1 for details).

differences in infection control, human population demographics, or environmental *P. aeruginosa* populations among Canadian provinces. Australian studies provide evidence that, except for known epidemic strains (17), CF strains are a sample of the environmental *P. aeruginosa* population (18, 19). More in-depth analyses are forthcoming for CF clinics where genomic data can be associated with patient identifier (ID), age, study time point, etc. Unfortunately, this type of information, although essential to direct further investigation of genomic data, proved extremely difficult to obtain.

Heterogeneity in *P. aeruginosa* population structure across Canada alone emphasizes the need for more customized patient care in the context of CF respiratory infections. This, of course, only adds to the great variability in antimicrobial resistance among *P. aeruginosa* isolates (11, 20). Canadian molecular epidemiology of *P. aeruginosa* may benefit from similar nationwide data from the United States. But, as mentioned in a recent review (16), there is a void to be filled in the literature in this regard.

Accession number(s). Assembled genomes used in this study are available as part of BioProject accession number PRJNA325248 (International *Pseudomonas aeruginosa* Consortium [IPC] genome sequencing project). The accession numbers for newly sequenced genomes are RWKX00000000 to RWVK00000000. The complete list of accession numbers is provided in Data Set S1 in the Supplemental Material.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.02019-18.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.
SUPPLEMENTAL FILE 2, XLS file, 0.1 MB.

ACKNOWLEDGMENTS

This work was supported by Cystic Fibrosis Canada (through CF Canada grant 2610 and a postdoctoral fellowship to J.J.).

S.J.C. is a research scholar of the Fonds de Recherche du Québec-Santé.

We thank Maryse Bandet, Annie Hallée, Cindy Lalancette, and David Lalonde Séguin for their assistance with isolate and data collection. We also thank members of the IBIS genomics analysis platform for their support.

REFERENCES

1. Rozen R, De Braekeleer M, Daigneault J, Ferreira-Rajabi L, Gerdes M, Lamoureux L, Aubin G, Simard F, Fujiwara TM, Morgan K. 1992. Cystic fibrosis mutations in French Canadians: three CFTR mutations are relatively frequent in a Quebec population with an elevated incidence of cystic fibrosis. Am J Med Genet 42:360–364. https://doi.org/10.1002/ajmg.1320420322.
2. Daigneault J, Aubin G, Simard F, De Braekeleer M. 1992. Incidence of cystic fibrosis in Saguenay–Lac-St.-Jean (Quebec, Canada). Hum Biol 64:115–119.
3. Laberge AM, Michaud J, Richter A, Lemyre E, Lambert M, Brais B, Mitchell GA. 2005. Population history and its impact on medical genetics in Quebec. Clin Genet 68:287–301. https://doi.org/10.1111/j.1399-0004.2005.00497.x.
4. Madore A-M, Prévost C, Dorfman R, Taylor C, Durie P, Zielenski J, Laprise C. 2008. Distribution of CFTR mutations in Saguenay– Lac-Saint-Jean: proposal of a panel of mutations for population screening. Genet Med 10:201. https://doi.org/10.1097/GIM.0b013e318164cb1c.
5. Parkins MD, Glezerson BA, Sibley CD, Sibley KA, Duong J, Purighalla S, Mody CH, Workentine ML, Storey DG, Surette MG, Rabin HR. 2014. Twenty-five-year outbreak of *Pseudomonas aeruginosa* infecting individuals with cystic fibrosis: identification of the Prairie epidemic strain. J Clin Microbiol 52:1127–1135. https://doi.org/10.1128/JCM.03218-13.
6. Speert DP, Campbell ME, Henry DA, Milner R, Taha F, Gravelle A, Davidson AG, Wong LT, Mahenthiralingam E. 2002. Epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis in British Columbia, Canada. Am J Respir Crit Care Med 166:988–993. https://doi.org/10.1164/rccm.2203011.
7. Aaron SD, Vandemheen KL, Ramotar K, Giesbrecht-Lewis T, Tullis E, Freitag A, Paterson N, Jackson M, Lougheed MD, Dowson C, Kumar V, Ferris W, Chan F, Doucette S, Fergusson D. 2010. Infection with transmissible strains of *Pseudomonas aeruginosa* and clinical outcomes in adults with cystic fibrosis. JAMA 304:2145–2153. https://doi.org/10.1001/jama.2010.1665.
8. Lyczak JB, Cannon CL, Pier GB. 2000. Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. Microb Infect 2:1051–1060. https://doi.org/10.1016/S1286-4579(00)01259-4.
9. Middleton MA, Layeghifard M, Klingel M, Stanojevic S, Yau YCW, Zlosnik JEA, Coriati A, Ratjen FA, Tullis ED, Stephenson A, Wilcox P, Freitag A, Chilvers M, McKinney M, Lavoie A, Wang PW, Guttman DS, Waters VJ. 2018. Epidemiology of clonal *Pseudomonas aeruginosa* infection in a Canadian cystic fibrosis population. Annals ATS 15:827–836. https://doi.org/10.1513/AnnalsATS.201801-007OC.
10. Somayaji R, Lam JC, Surette MG, Waddell B, Rabin HR, Sibley CD, Purighalla S, Parkins MD. 2017. Long-term clinical outcomes of 'Prairie epidemic strain' *Pseudomonas aeruginosa* infection in adults with cystic fibrosis. Thorax 72:333–339. https://doi.org/10.1136/thoraxjnl-2015-208083.
11. Freschi L, Jeukens J, Kukavica-Ibrulj I, Boyle B, Dupont M-J, Laroche J, Larose S, Maaroufi H, Fothergill JL, Moore M, Winsor GL, Aaron SD, Barbeau J, Bell SC, Burns JL, Camara M, Cantin A, Charette SJ, Dewar K, Déziel E, Grimwood K, Hancock REW, Harrison JJ, Heeb S, Jelsbak L, Jia B, Kenna DT, Kidd TJ, Klockgether J, Lam JS, Lamont IL, Lewenza S, Loman N, Malouin F, McArthur AG, McKeown J, Milot J, Naghra H, Nguyen D, Pereira SK, Perron GG, Pirnay J-P, Rainey PB, Rousseau S, Santos PM, Stephenson A, Taylor V, Turton JF, Waglechner N, Williams P. 2015. Clinical utilization of genomics data produced by the International *Pseudomonas aeruginosa* Consortium. Front Microbiol 6:1036. https://doi.org/10.3389/fmicb.2015.01036.
12. Jeukens J, Freschi L, Vincent AT, Emond-Rheault JG, Kukavica-Ibrulj I, Charette SJ, Levesque RC. 2017. A pan-genomic approach to understand the basis of host adaptation in *Achromobacter*. Genome Biol Evol 9:1030–1046. https://doi.org/10.1093/gbe/evx061.
13. Inouye M, Dashnow H, Raven L-A, Schultz M, Pope B, Tomita T, Zobel J, Holt K. 2014. SRST2: rapid genomic surveillance for public health and hospital microbiology labs. Genome Med 6:90. https://doi.org/10.1186/s13073-014-0090-6.
14. Jolley K, Maiden M. 2010. BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics 11:595. https://doi.org/10.1186/1471-2105-11-595.
15. Romling U, Kader A, Sriramulu DD, Simm R, Kronvall G. 2005. Worldwide distribution of *Pseudomonas aeruginosa* clone C strains in the aquatic environment and cystic fibrosis patients. Environ Microbiol 7:1029–1038. https://doi.org/10.1111/j.1462-2920.2005.00780.x.
16. Parkins MD, Somayaji R, Waters VJ. 2018. Epidemiology, biology, and impact of clonal *Pseudomonas aeruginosa* infections in cystic fibrosis. Clin Microbiol Rev 31:00019-18. https://doi.org/10.1128/CMR.00019-18.
17. Kidd TJ, Magalhães RJS, Paynter S, Bell SC. 2015. The social network of cystic fibrosis centre care and shared *Pseudomonas aeruginosa* strain infection: a cross-sectional analysis. Lancet Respir Med 3:640–650. https://doi.org/10.1016/S2213-2600(15)00228-3.
18. Kidd TJ, Ritchie SR, Ramsay KA, Grimwood K, Bell SC, Rainey PB. 2012. *Pseudomonas aeruginosa* exhibits frequent recombination, but only a limited association between genotype and ecological setting. PLoS One 7:e44199. https://doi.org/10.1371/journal.pone.0044199.
19. Ranganathan SC, Skoric B, Ramsay KA, Carzino R, Gibson A-M, Hart E, Harrison J, Bell SC, Kidd TJ. 2013. Geographical differences in first acquisition of *Pseudomonas aeruginosa* in cystic fibrosis. Ann Am Thorac Soc 10:108–114. https://doi.org/10.1513/AnnalsATS.201209-077OC.
20. Jeukens J, Freschi L, Kukavica-Ibrulj I, Emond-Rheault J-G, Tucker NP, Levesque RC. 2019. Genomics of antibiotic-resistance prediction in *Pseudomonas aeruginosa*. Ann N Y Acad Sci 1435:5–17. https://doi.org/10.1111/nyas.13358.
21. Freschi L, Vincent AT, Jeukens J, Emond-Rheault J-G, Kukavica-Ibrulj I, Dupont M-J, Charette SJ, Boyle B, Levesque RC. 2019. The *Pseudomonas aeruginosa* pan-genome provides new insights on its population structure, horizontal gene transfer and pathogenicity. Genome Biol Evol 11:109–120. https://doi.org/10.1093/gbe/evy259.